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TRANSPOSABLE ELEMENTS IN LEPIDOPTERA: HOBO-LIKE TRANSPOSONS IN *HELIOTHIS VIRESCENS AND HELICOVERPA ZEA*

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Summary: In the present study, a PCR-based approach was undertaken to determine if members of the hobo-like family of transposable elements were identifiable within the genomes of a number of diverse species, including *Heliothis virescens* and *Helicoverpa zea*. The amplified products derived from both *H. virescens* and *H. zea* were cloned and characterized. Analysis of the DNA sequence and the single open reading frame found within these fragments clearly demonstrates that these elements are closely related to the hobo transposon from *Drosophila melanogaster*. In addition, amino acid sequence analysis of the members of this family defines consensus, specific amino acids found within similar regions of all members of this transposon family.

The tobacco budworm (*Heliothis virescens*) and the corn earworm (*Helicoverpa zea*) are pests on a wide variety of crops. To date, their control has been unsuccessful, and has been attempted almost exclusively through the use of synthetic organic pesticides, underscoring the need for the development of safe and effective alternative control methods. This has stimulated interest in the potential use of genetically engineered insects as biological control agents. The use of such a program depends on the ability to transfer DNA molecules into the genome of the host insect. Vector transformation systems using transposable elements appear to the method of choice, since DNA introduced via a transposon vector system can be randomly and stably inserted into the recipient genome without necessarily adversely affecting the expression of other genes. Unfortunately, insect gene-

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transformation systems are only available in *Drosophila*, thereby emphasizing the need to identify possible vectors which may be of use in insects of agricultural importance. In *Drosophila*, the two most exploited vector systems involve the use of the P and the hobo elements (1,2), however the usefulness of these two systems outside of the species is somewhat in doubt. It has clearly been demonstrated that the P element is quite species specific and basically not functional outside of *Drosophila*(1). Recently, Calvi *et al.*(3) found that the polypeptide sequences of the putative transposases of three very distinct members of this family (Activator [Ac] from *Zea mays*, Tam3 from *Antirrhinum majus*, and hobo from *Drosophila melanogaster*) have several regions of significant amino acid sequence similarity and homology, suggestive of a horizontal gene transfer event between the plant and animal kingdoms. Therefore, it was of both academic and practical interest to determine if similar transposable elements could be identified within the genomes of *H. virescens* and *H. zea*.

Materials and Methods

The degenerate PCR primers were: 1) Forward, GGACNGTIGATATGTGG and 2) Reverse, GGXGTGCTATCCCAIGGIGT, where I represents inosine(4). The thermo-cycling reaction was performed on a Perkin Elmer thermal Cycler 480, and the conditions were as follows: 94°C for 2min, ramp to 39°C over 30 sec., hold at 39°C for 1min, ramp to 72°C over 2min, and hold at 72°C for 3min. This was performed for 35 cycles with a final extension at 72°C for 7 min. Cloning of amplified products was accomplished by isolating amplified products from agarose gels by the freeze/squeeze method (5). Isolated fragments were reamplified under identical conditions, and the resultant products again isolated from agarose gels and used directly with the PGEM-T cloning system (Promega, Madison WI). All other molecular biological techniques were performed as described previously (6). Plasmid DNA from the positive transformants (white colonies) were prepared by the alkaline minilysate procedure (7) and sequenced directly using a variation of the dideoxy chain termination system of Sangar et al., (8) which utilizes taq DNA polymerase.

Results

Identification of hobo-like elements

While a number of different approaches have been utilized for the identification and cloning of transposon and transposable-like sequences from a variety of insects(1), the simplest and most direct appears to be searching for sequence homology using either direct hybridization or the polymerase chain reaction. In fact, the latter approach has proven quite successful for the mariner family of elements(9). To determine if a similar approach could be undertaken to identify members of the hobo element family of elements, DNA from a number of different organisms were subjected to amplification using the described primers. The primers correspond to region 1 and region 2 as

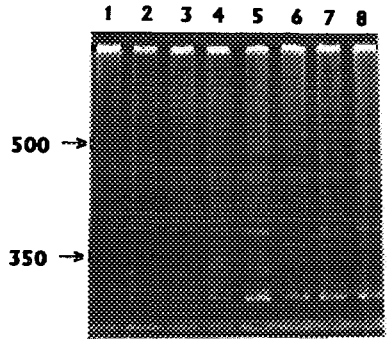

FIGURE 1. PCR AMPLIFIED HOBO-LIKE ELEMENTS IN VARIOUS SPECIES.
PCR-generated fragments using degenerate hobo primers. Lane 1, no DNA (negative control); lane 2, *Archytas marmoratus*; lane 3, *Chirronomous tentans*; lane 4, cricket; lane 5, western corn root worm; lane 6, *D. melanogaster*; lane 7, *Heliothis virescens*; lane 8, *Helicoverpa zea*.

described by Calvi et al. (3). Appropriately sized bands were observed in amplified DNA from: western corn rootworm, *D. melanogaster*, *H. zea* and *H. virescens*, thus demonstrating that 1) these primers were capable of amplifying sequences found within diverse organisms, and 2) suggesting that members of the hobo family of transposable elements may be broadly distributed in nature (Figure 1). Since our primary interest lies in identifying lepidopteran transposons for the development of vector systems, we chose to clone and analyze the fragments from *Heliothis virescens* and *Helicoverpa zea* for further characterization.

DNA sequence determination

Since a number of DNA fragments corresponding to the appropriate size were observed, all were cloned and sequenced. The DNA sequences of several independent clones were determined and found to contain the same DNA sequence, thereby confirming the fidelity of the Taq polymerase used. Those sequences not capable of encoding a continuous open reading frame were not further analyzed. The actual size of the *Heliothis virescens* (designated HVHLE) insert is 373bp while the *Helicoverpa zea* (designated HZHLE) insert is 376bp. The consensus DNA sequences of both fragments is presented in Figure 2. There are several regions of potential secondary structure, and both contain a single continuous open reading frame. It is particularly noteworthy that the areas of homology between these sequences closely correspond to the region responsible for encoding the transposase function of the *D. melanogaster* element (3). Alignment of both HVHLE and HZHLE DNA sequences with

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HZHLE   TCG GAC TCT TAC CAG CGA GAG TTC TAT ATT TCT GGC GTC ACA TTT
HVHLE   GTG GAC CAT TAC CAG AAA GAG TTC AAG GCT TAT ATC GTC ACA TTT

HZHLE   CAT TCG GAC AAA AAA AAT CGT TTC TGC GTA TAC ATT GTT GGC TTA
HVHLE   TGT GTG ATG AAA GCA ACG AGT CAG TGC GTA TAC ATT GTT GGC TTA

HZHLE   AGG CAG AAT TCG GTA AGA AAA CAG ACG ACG TCA CTA GAA AAA ATC
HVHLE   AGG CAG AAT GGG ATA AGA --- CAG AGA ACG TGC ATA GAA AAA ATC

HZHLE   GAA AAA ATA AAA GGA GTA CGG GCT GCA CGT GCA CTG TTC TCA AAA
HVHLE   GAA AAA ATA AAA GGA GTA TAC CAT GCA CGT GCA CTG TTC TCA AAA

HZHLE   AAA AAA TCT GTT ATT ATT AAA GCT AAT CGG ATA AAG AAA ATT GTG
HVHLE   AAA AAA TCT GTT ATT ATT AAA GCT AAT GAA ATT AAA AAA ATT GTG

HZHLE   AAA CTG CAC CTA CAA GTA TTA CAG CTT CTC AGG GAC GAC ATT CGG
HVHLE   AAA ATA CAC CTA CAA GTA TTG CAG CTT CTC AGG AAA ACA ATT TGT

HZHLE   GGT GGT GCA GAA GTC AAA AAA ATA GTA AAA AAA TCT TCT CCA CTA
HVHLE   TTT GGT GCA GAA GTC AAA AAA ATA GTA AAA AAA TCT TCT CCA CTA

HZHLE   CAA CTA AAA TGT --- GAC CTA GAC AGC --- ---
HVHLE   CAA CTA AAA TGT GCA GAC CTA GAC AGC TAT GTT
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FIGURE 2. DNA HOMOLOGY BETWEEN LEPIDOPTERAN HOBO ELEMENTS.

DNA sequence homology between *H. zea* and *H. virescens* hobo elements. The *H. zea* element is designated HZHLE while the *H. virescens* element is HVHLE. Identical nucleotides are indicated by [:] between the sequences.

other known transposon-like sequences revealed only minimal homologies, in a fashion similar to that seen with hobo (data not shown).

HVHLE open reading frame resembles transposases

Translation of the HVHLE and HZHLE DNA sequences revealed single, continuous open reading frames. When these putative polypeptides are aligned (allowing for gap correction) with the transposases from the *D.melanogaster* hobo element, the *Zea mays* Activator element, and Tam3 from *Antirrhinum majus* there is a high degree of amino acid identity observed over the entire length of these elements, with the most homologous being between HVHLE and HZHLE. Figure 3 presents these homologies in four arbitrary groupings. For the sake of establishing a consensus hobo-like sequence, percent homology is defined only for those regions showing identity between HVHLE and HZHLE. Gaps have been introduced to provide for

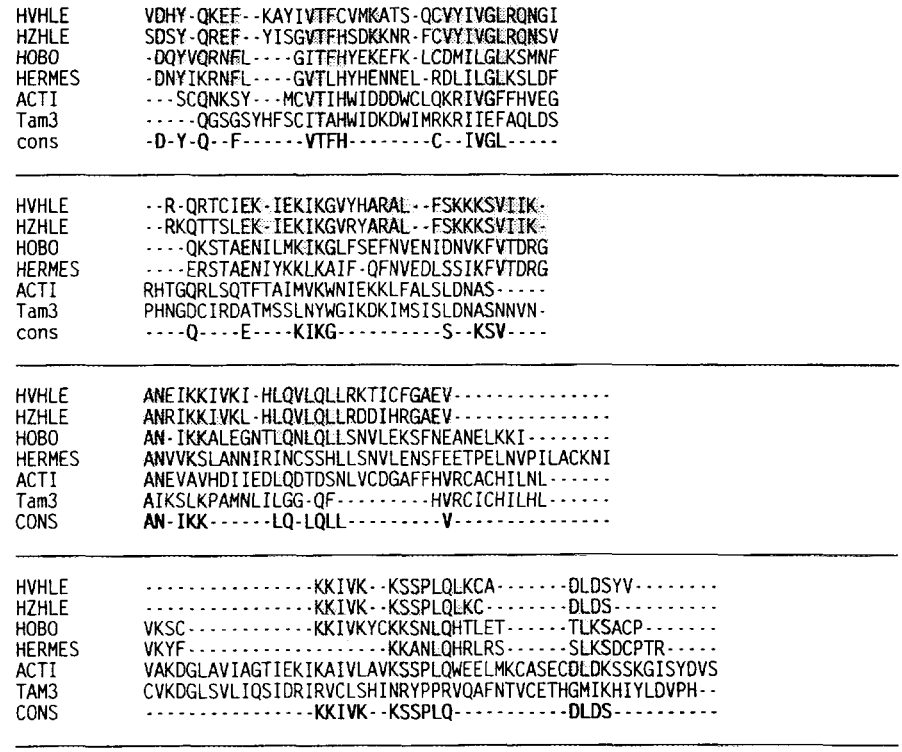

FIGURE 3. AMINO ACID ALIGNMENT OF HOBO-ELEMENT FAMILY MEMBERS.

The amino acid sequences of HVHLE, HZHLE, Hobo, Hermes, Activator, and Tam3 were aligned using the HBIOI Prosis protein analysis program. Gaps were introduced such that those regions previously described as being homologous were properly aligned(7,14). Those regions showing exact amino acid identity to both HVHLE and HZHLE have been shaded. HVHLE represents the hobo-like element from *Heliothis virescens* and HZHLE, that from *Helicoverpa zea.* HOBO, HERMES, ACTI, and TAM3 correspond to the elements from *D. melanogaster*, *Musca domestica*, *Z. mays*, *A. majus.* The consensus sequence constitutes those identical amino acids found in common by at least three of the six members of the family. Regions I, II, III, and IV were chosen arbitrarily.

previously published alignments for Tam3, Activator, and hobo (3, 11). It is particularly interesting to note that between HZHLE, HVHLE, and hobo, regions of identity appear in blocks possible suggesting functional domains of the putative proteins. This is also supported by a 64.3% functional amino acid homology between hobo and the two lepidopteran transposases, stretching across the entire polypeptide, clearly demonstrating the relatedness of these proteins. It is also noteworthy that there is only limited homology between HVHLE, HZHLE and either Ac(11) or Tam3(12), two other members of this family suggesting that, in a manner similar to that seen for members of the mariner family of elements(9,10), hobo-like family members may prove to be quite divergent. When these data are taken together, it strongly suggests that the

cloned fragments are, indeed, the internal coding regions of lepidopteran-derived transposable elements, and that these elements are members of the hobo-element family of transposons.

Discussion

The transposable element *hobo* was originally described by McGinnis *et al.*, (13) from an insertional mutant of a glue protein in *D. melanogaster*. In the genus *Drosophila*, DNA sequences hybridizing to *hobo* probes have been found to have an extremely limited occurrence among species(14). In this report, hobo-like sequences were identified in the lepidopteran species *H. virescens* and *H. zea*.

Two required components of a transposon-based management program are 1) the element must be widely distributed in nature such that transposable immunity is prevalent, and 2) a laboratory strain must exist which has never seen the transposon and, therefore can act as a recipient for any DNA to be inserted. Analysis of the single translated product encoded by these lepidopteran elements revealed significant functional amino acid homology with the hobo element from *D. melanogaster*. Furthermore, PCR amplification of other species showed appropriately sized amplified fragments, thereby suggesting the successful utility of a hobo-like element in biotransformation. Additional analyses of captive populations will reveal if, indeed, a receptive strain is presently available.

It should also be noted that the area of homology shared among these proteins corresponds to a region located within the C-terminal two thirds of the polypeptide, thus suggesting a related function within this region. Although several different transposases have been shown to be DNA binding proteins (12, 13), the basic DNA binding domain has been shown to reside within the first 100 amino acids, a region showing no significant homology among any of the proteins analyzed(12). It could be easily imagined that specific regions of these proteins could be involved in nucleotide binding, DNA insertion, or even DNA target-site juxtaposition. Further analysis of the transposition process may now be accomplished by altering those amino acids found to be common among hobo-element family members.

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